Synergistic Antibacterial Effects of Probiotic LAB and It's Bioactive Molecules with Turmeric Powder as Synbiotic against Dermal Infection.

A synopsis submitted to

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Nanded (Maharashtra)

For

Registration to Doctor of Philosophy (Microbiology)

Under the faculty of Science

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AUG 2021

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Introduction:

The origins of dairy products are derived from the beginning of civilization; they are mentioned both in the Bible and in Hindu scriptures. The concept of Probiotic was introduced in the early twentieth century by Russian scientist and Nobel laureate Metchnikoff. The current definition of probiotics is that, a living microorganism that, when administered in the right doses, provides health benefits to the host (FAO / WHO 2002). This definition was retained by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013. Finally, in 2007, FAO / WHO experts described prebiotics as an incomparable dietary supplement that provided health benefits to the host with microbiota modulation. Features of probiotics include tolerance of acidic pH, concentration of bile salts, NaCl, pancreatic enzyme to prevent dysbiosis and development of immune system, health. Other selected species of Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus and Saccharomyces have been developed in food products because of their known health benefits. Probiotics, especially Lactobacillus and Bifidobacterium have been associated with reduced lactose intolerance and treatment of viruses, bacteria and viruses or radiotherapy that has caused diarrhea immunomodulation antimutagenic and anticarcinogenic effects and lowering blood cholesterol.

Prebiotics can be used as an alternative to probiotics or as an additional supplement to them. Prebiotics have great potential for altering the microflora, but these mutations occur at the level of species and species and do not readily predict priori. skin pH, plays an important role in determining the competitive outcome of interspecies. Both for the sake of efficiency and safety, the development of prebiotics aimed at benefiting human health should take into account the profiles of potential species of animals. Potential sources of Probiotics: tomatoes, artichokes, bananas, nutritious vegetables, berries, garlic, onions, chicory, raw vegetables, cereals, and oats, linseed, barley and wheat. Some prebiotics are synthetically produced, among others: lactulose, galactooligosaccharides, fructooligosaccharides, maltooligosaccharides, cyclodextrins, and lactosaccharose. Fructans, such as inulin and oligofructose, are believed to be the most widely used and effective against many types of probiotics. The plant prebiotic Turmeric (Curcuma longa) rhizome powder is best known for its anti-inflammatory, anti-bacterial and antioxidant activities.

In 1995, Gibson and Roberfroid introduced the term "synbiotic" to describe a combination of active probiotics and prebiotics. As the term "synbiotic" means interaction, this term should be applied to those products where the prebiotic component selects the probiotic microorganism (Cencic A., Chingwaru W., et al. 2010). Further research on the discovery of new probiotic species, selection of probiotics and prebiotics synbiotics, dose setting, safety of use, and clinical trials documenting the desired health outcomes are needed. Results should be confirmed in well-organized clinical trials conducted by private research institutes.

Next world is probiotic. Antimicrobial activity against human as well as mammal pathogens which are live on the skin surface. The optimism associated with probiotics, however, contradicts doubts as many "probiotic" products on the market are reliable in content and have no clinical evidence. In some village butter probiotic with haldi powder use as antiseptic, bactericidal against a pathogenic strain as a result cure the skin infection. The alternative of antibiotics is a

probiotic, which are produced the bioactive compound. Some pathogenic strains are resistance against antibiotics, probiotic use as alternative bio-therapeutic agent of medicine (pharmaceutical, veterinary, cosmetics). It has been observed that existing cosmetic products and their ingredients make use of viable cultures of probiotics and/or their lysates for their production to maintain good skin health. Properties of some probiotics or their lysates, their incorporation in certain cosmetics have been proposed in order to achieve more radiant looking skin. The aim of this study will be to investigate the antibacterial synergistic activities of probiotic lactic acid bacteria (LAB) and Turmeric powder as synbiotic against Dermal infection.

Review of Literature: -

International: -

The L. acidophilus A001F8 with prebiotic CLE (Curcuma longa rhizome Extract) has a synergistic effect of antibacterial activity against C. acnes, and thus, that prebiotic CLE can be utilized for the development of cosmetics and medicines for the treatment of acne (Jina Kim, Hyaekang Kim et al.,2020). Various cosmetic products which are alter the microbiome of the skin and decrease the beneficial microbial loads that result causes diseases in large organ of the skin. In recent years, the use of probiotics has become an interesting area due to applications in various fields such as diary, food and beverage, agriculture, health care and aqua culture (Zielinska and Kolozyn-Krajewska., 2018). Although reducing acne symptoms with lactobacilli in topical cream can be as effective as cosmetics (Lebeer, S.; Oerlemans, E.; Claes, I.; 2018). Probiotics stimulate immune function, suppress viral infections, remove carcinogens, improve calcium absorption and reduce faecal enzyme activity (Ouwehand et al., 1999; Zubillaga et al., 2001; Holzapfel & Schilling 2002). Probiotics have a significant impact on the therapeutic context as they will be aimed at reducing the use of antibiotics and thus reducing the side effects associated with their chronic use (Vasiriki Lolou et al., 2019). Fermentation can be an aerobic or anaerobic process in which a living thing (or its enzymes) chemically converts a substrate to produce an interesting product (Pohorecki, R., Bridgewater et al.,).
Microbiota dysbiosis, whether in the intestine and / or other tissues, is closely related to the prevention and / or treatment of diseases, inflammatory diseases, autoimmune diseases, cancer, neurodegenerative diseases, depression, anxiety, diet, trauma, metabolic syndrome and related diseases among other disorders (Kang and Im, 2015; Sander, 2017; Westfall et al., 2017; Lee et al., 2018). Interactions can occur when lactic acid starches and probiotic bacteria are mixed to form dairy products. Several types of interactions have been found among lactic acid starter bacteria (C. G. Vinderola, P. Mocchiutti, 2002). Both L. plantarum ne-L. helveticus are tolerant of low pH, high bile salts, and concentration of NaCl and this bacterium has anti-bacterial activity against food-borne microorganisms and was resistant to many antibiotics (Mahreen Ul Hassan et al., 2020).

National: -

Strain S. lavendulocolor VHB-9 cultural parameters required for optimal yield of bioactive metabolites, as well as chemical breakdown of compounds were also investigated (Vijayalakshmi et al.2018). Probiotic Power of Lactobacillus Strain by antimicrobial activity

against certain human pathogenic species (Parisa S, et al., 2014). Probiotic adhesion and colonization of the intestinal epithelium, stressful conditions in the intestinal tract, anti-bacterial activity against pathogenic strains, immune function, biological activity in the GI tract and ultimately health benefits for the manager (Misra et al.2019; Fenster et al. al. 2019; Sanders et al. 2019). Lactobacillus GG and L. rhamnosus GG reduces symptoms of food allergies while at the same time plays an important role in reducing the risk of developing autoimmune disease (Manoj G et.al., 2012). Probiotics produce a few bioactive compounds that have beneficial effects in fighting GIT infections, which contribute to homeostasis and competitive release of viruses (Verma and Shukla, 2013). Probiotics promise another treatment with minimal effect on commercially available antibiotics (Andrew Pradeep M, et al., 2017). No doubt we will see a significant increase in the role of probiotics in diet and medicine in the coming decades.

Importance of Probiotics*:*

1. Probiotic and Allergy
2. Probiotics and Blood Pressure
3. Probiotics and Inflammatory Bowel Disease
4. Probiotics and Urogenital infections (Bacterial vaginitis)
5. Probiotics and Liver Diseases
6. Probiotics and Cholesterol Assimilation
7. Probiotics and Dental Caries
8. Probiotics and Orthodontic Treatment
9. Probiotics and Oral Health
10. Probiotics and Voice Prosthesis
11. Probiotics and Halitosis
12. Probiotics and Periodontal Diseases
13. Immunologic Enhancement/ Immunity Stimulation
14. Probiotics and HIV
15. Probiotic and Prebiotics on Skin Disease
15.1. Dermatitis
15.1.1. Atopic Dermatitis (AD) or atopic eczema
15.1.2. Allergic Contact Dermatitis
15.2. Skin Infections
15.2.1. Wounds
15.2.2. Acne
15.3. Psoriasis
15.4. Photoaging

Aim and Objectives:

A) To Collect milk sample.
B) To isolate beneficial microorganism (Lactobacillus sp.,) from collected samples.
C) To study and identify the biochemical characteristics of isolated samples.
D) To optimize the Physico-chemical Parameters of the isolates.
E) To study Probiotic / lysates against pathogenic strain**.**
F) To study synergetic action of probiotic butter with certain natural ingredients to treat dermal problems.
G) To study synergetic action of probiotic against antibiotic resistance bacteria.

Year wise Plan of work and target to be achieved

First Year (2021-2022): -

- Completion of course works and Literature survey.
- Sample collection.
- Isolation of LAB.

Second year (2022-2023): -

- Isolation and identification of Lactobacillus sp., in MRS Media.
- Screening of isolated LAB.
- Production of Bioactive compound
- Purification and characterization of produced Bioactive compound.
- Antimicrobial activity test by Kirby-Bauer Disc diffusion method.
- Probiotic action of different antibiotic resistant bacteria will be studied.

Third year (2023-2024): -

- Molecular identification of potential isolate.
- Probiotics with phytochemical of turmeric study will be done on pathogenic strain of dermal.
- Result will be completed.
- Preparation and submission of research article to the reputed national and international journals.

Fourth year (2024-2025): -

- Final synopsis will be prepared.
- Thesis writing
- Submission of thesis to the university

Materials and Methods:

Probiotic Source:

Total of 2 probiotic sources Lalkandhari cow curd and buffalo curd – sample will be collected from the rural area. Samples consisted of two groups: group I, Lalkandhari cow curd; group II, buffalo curd. Sample group I and group II will take weighed10gm.

Isolation and purification of lactic acid bacteria from sample:

For LAB classification, a serial dilution agar method will be used. Ten grams of each sample (excluding 10 ml curd) will dissolve into 90 ml of MRS broth. After melting in the MRS broth they will move in the same direction and will be heated to 37 ° C for 24 hours in aerobic state. In the serial dilution agar plate system, 10ml of the stock solution was suspended and stirred in 90 ml water spaces to form a microbial suspension. A serial purification of 10-2, 10-3, 10-4, 10-5 and 10-6 is performed by inserting a 10ml pipe into 90ml water spaces. 10 ml of each dilution will be injected into MRS agar plates (prepared by pouring 15ml of sterile fermented cooling sprays) and incubated at 37oC for 24 hours to grow germs. Plates were recognized for colonial appearance and the number of colonies produced on each plate of different dilution was obtained. Pure culture was obtained. Microscopic and Biochemical confirmation was done and culture stored at 4oC (Andrew Pradeep M, et al.,2017).

Evaluation of probiotic potentials of isolated bacterial cultures:

Determination of probiotic potential as follows:

Temperature sensitivity:

Cultures of selected bacteria are grown at various temperatures, i.e., 25o, 30o, 37oand 40oC for 48-72 hours. The 0.1ml inoculum was then transferred to MRS plates in the form of a drain plate and stored at 37oC for 48 hours. The growth of LAB on MRS agar plates was used to select isolate as a temperature tolerant (Tamber, D.H, et al., 2010).

Lactose utilization:

Acid production by selected bacterial cultures was obtained by observing the color change of the medium. The sterilized fermentation medium (10g peptone, NaCl 15g, red phenol 0.018g, lactose 5g, 1L of pure water and final pH 7.0) was vaccinated by different cultures and incorporated into -35oC 24-48 hours. The transition from red to yellow indicates acid production (Ahmed, T.et al., 2004).

NaCl tolerance:

The salt tolerance of selected bacterial cultures was tested 3 days after incorporation of 1-6.5% NaCl concentration in the MRS broth (Adebayo-tayo, et al., 2008). Controversial functions A disk distribution method was used to obtain antimicrobial activity as described above.

Organic Acid Production Profile:

Examination of organic acid production by LAB species was in the manner described by Erwin et al. [25] and changes. Overnight (incorporated at 37∘C in anaerobic pot (Oxoid, UK) containing gas packet (AnaeroGen, Oxoid, UK, oxygen level <1%, CO2 level between 9 and 13%) for each type centrifuged at 1500 × g 10 minutes at room temperature and 3mL of supernatant added to 600 μL of 24% metaphosphoric acid (at 3N H2SO4). The mixture is stored at room temperature 24 h then centrifuged at 1500 × g for 20min at room temperature. 0.5mL of 20mM 4-methylvaleric acid, and 1 μL of each sample. × 25 μm, internal diameter) The column temperature is set at 100 to 190∘C with a temperature range of 5∘C / min increments for better division. Oven temperatures in the oven, FID, and injector were 160, 250, and 230∘C, respectively. Nitrogen with a flow rate of 1.0 mL / min was used as the company's gas. The internal level was 20mM4-methylvalericacid. Acetic acid (20 mM), propionic acid (10 mM), butyricacid (10mM), isobutyric acid (10 mM), valeric acid (10 mM), isovaleric acid (10mM), and and 4-methylvaleric acid (10mM) have been used as standard solutions for peeling. Fornonvolatile fatty acids (lactic and succinic acids) (non-VFA) (non-VFA), 0.5mL of supernatant were added with 0.5mLof 20mM fumaric acid and 1 μL of each sample injected into GC. Oven temperatures, FID, and injections were 180,150, and 110∘C, respectively. Nitrogen with a flow rate of 1.0 mL / min was used as the company's gas. The internal level was 20mM fumaric acid. Lactic acid (20 mM), succinic acid (10mM), and fumaric acid (10mM) have been used as standard solutions for peeling. Tests were performed twice, each three times.

Screening of Test microorganisms:

Escherichia coli, Proteus sp, Klebsiella sp and two Gram-positive *Staphylococcus epidermidis* and, *Staphylococcus aureus, Salmonella typhi, Candida albicans, etc.*

Standardization of test microorganisms:

Tested microorganisms will be synthesized using standard 0.5 McFarland. The McFarland Standard will be used as a reference to adjust the suspension of germs so that their number is within the given range. 0.5 McFarland provides approximately 1.5 x 108 CFU / ml cell density, absorbing 0.132 at a wavelength of 600 nm. Microbial suspension will be adjusted to your sterile

food broth and compared with appearance or moderate absorption to normal (Andrews, et al., 2001).

Purification of the Bioactive Compounds

One-night stand-alone cultures were planted in MRS broth (10% inoculums (108 CFU / ml) and fitted with a 72hin shaker incubator (200rpm) at 37 ° C. Bacterial suspensions of each type were centrifuged at 12000rpm for 15 minutes and the supernatant was used as a raw bioactive compound. The supernatant was separated and filled with 70% ammonium sulfate and stored at 4 ° C to reduce protein. The pellet was collected after centrifugation at 12000 rpm at 4 ° C for 25 minutes and dissolved in phosphate buffer (0.1M, pH 7.0) and distilled in the same machine at 4 ° C on the night of Ogunbanwo et al. [3]. Dialyzed proteins used in the Sephadex G-100 column (1.6 × 36cm), pre-mixed with phosphate buffer (pH 7.0) and components showing high bioactive compound activity were collected and included in chemical analysis.

Chemical Analysis of the Bioactive Compounds by SDS-PAGE and HPLC

The molecular weight of the purified bioactive compound was determined by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to a procedure described by Laemmli in 1970. The electrophoresis gel was coated with Coomassie Brilliant Blue R-250 and washed with a mixture of acetic acid-methyl alcohol-water (5: 5: 1 v / v). Low molecular weight marker has been used as a standard marker (Genei, India). To make HPLC, bioactive compounds were incorporated into 12000rpm for 15 minutes. The supernatant was then filtered through a strain of 0.22µm and injected into the HPLC system. Analysis of bioactive compounds was performed by the HPLC system (Shimadzu, Japan) with a high-frequency UV-VIS detector. The HPLC column was C18, 250 × 4.6mm, particle size 5µm, Solar Fire and moving phase of Chromatographic conditions was phosphate buffer 0.01 mol / L-3.0% methanol, pH 3, acquired at 210nm of Ultraviolet (UV) absorption, 0.7 mL / min flow rate and injection volume 10µl. Column temperature kept cool. The cell section was adjusted and filtered through a 0.22µm filter layer.

Screening of isolated bacterial cultures for antimicrobial activity by Kirby-Bauer method:

To test the probiotic bacterial culture will be injected into MRS broth soaked in 370C 24-48 hours in a shaker to make the fermentation process. After incubation, 2 ml of each concentrated broth and supernatant will be taken to test the antimicrobial activity in the form of Disc diffusion against six experimental microorganisms. Each individual culture will be testing all the experimental microorganisms. The night culture of pathogens planted in them respectively at 37 °C will be purified to a turbidity equal to 0.5 McFarland standard.

Determination of Growth Curves of Probiotics with and without Turmeric

Lactobacillus sp. (L. acidophilus A001F8, L. rhamnosus A001G8, L. paracasei A002C5, L. plantarum A003A7 and L. casei A003D4) with and without 0.01%CLEwere cultured at 37 _Cfor 18 h. The absorbance was measured by a UV-spectrophotometer (Optizen POP, Mecasys, Korea) at 600 nm (OD600) every 2 h for 18 h. To ensure accuracy, each sample was ten-fold serially diluted with PBS (Jina Kim, Hyaekang Kim et al., 2020).

Evaluation of antimicrobial activity of bioactive compound:

The antibacterial properties of both cells and the cell-free supernatant (CFSs) of the probiotic Lactobacillus species have been evaluated in a well-propagated way by small mutations. The night-grown culture of all species will be vaccinated in MRS broth and kept for 18-20 hours at 37 ° C. Older cultures will be harvested by centrifugation at 10000 g for 10 minutes at 4 ° C and supernatants without 200 µl cells used for testing. The antagonistic profile of bacterial isolate was studied to detect inhibitory activity against common E-bacteria. coli, Bacilluscerus, S. aureus, S. typhoid, Aeromonashydrophila and Klebsiella pneumonia. 300 µl ounces are mixed well with 10 ml of soft luria of Luria Broth (LB) and covered on a base plate of LB and left to harden. After hardening, the holes were bored on the plate with the help of pipette tips (200 µl, diameter 0.7 cm). The holes were then closed by adding 1-2 drops of soft agar to prevent leakage of supernatant years. Then 100 µl of prepared supernatant was added to each source and stored in the refrigerator for 1-2 hours to dissipate maximum energy. Test plates are incubated for 6-7 hours at 37 ° C. Prevention area is measured with the help of a site-measurement scale (Kaushik JK, Kumar A et al., 2009).

Detection of Bacterial Interactions (Growth Kinetics):

According to the results obtained by the screening method described above, growth kinetics were performed. The strains were grown at 37°C in broth in the presence (test curve) or not (control curve) of a concentrated cell-free supernatant (**CCFS**) of another species. The CCFS were obtained as described above, but before passage through the filter, they were fivefold concentrated (4 h, 43°C, under vacuum) so its addition (5% vol/vol) to the broth would not significantly dilute it. Growth kinetics were performed by optical density (560 nm) measurements. Viable cell counts were carried out in MRS agar (3 d at 37°C, aerobiosis for lactobacilli and anaerobiosis for bifidobacteria) or Elliker agar (3 d at 37°C, aerobiosis for streptococci) after 24 h of growth to estimate the differences in cell counts between test and control kinetics. Experiments were carried out in triplicate.

Antibiotic Susceptibility Test (Minimum Inhibitory Concentration):

Antibiotic exposure to LAB strains was performed using the broth micro dilution method according to ISO 10932 / IDF 233 [20] with few mutations. The antibiotics tested were ampicillin, clindamycin, gentamicin, streptomycin, tetracycline, erythromycin, kanamycin, and chloramphenicol (Sigma, USA). All antibiotics are dispersed to prepare stock solutions of 1280 μg / mL. Stock solutions were diluted in LAB susceptibility test medium (LSM) to obtain concentrations with a concentration of 0.25 to 128 μg / mL. To prepare the bacterial mucus, the colonies from the night culture of each type of LAB were suspended in 5mL 0.85% NaCl solution, adjusted to a turbidity of 0.2} 0.02 (OD620nm), and diluted by 1: 500 in LSM broth. Then, 50 μL of purified inoculum was added to each well of 96 small purifying plates containing 50 μL of antimicrobial solution, resulting in a combination of 5 logs of CFU / source in each inoculum of bacteria. Incubated plates are anaerobically placed at 37∘C for 48 hours. After incubation, low inhibitory concentration (MIC) values were determined as low antibiotic concentrations in which apparent growth was inhibited and compared with MIC breakpoint values for hetero-fermentative lactobacilli recommended by the European Food Safety Authority (EFSA) Panel. Supplements and Products or Materials Used in Animal Feed [22]. The accuracy of the test was assessed using the same type of quality control (Enterococcus faecalis ATCC 29212) as suggested by the Clinical and Laboratory Standards Institute (CLSI).

Arginine Hydrolysis Test:

For experiments with arginine hydrolysis, MRS broth (excluding glucose and extracted meat, mixed with 0.3% arginine and 0.2% sodium citrate) was used.

Butter LAB with Turmeric Powder Test on pathogenic organism:

Naturally probiotics present in butter; example Lactobacillus spp., Bifidobacteria, etc is antagonist/antimicrobial effect against the human and mammalian pathogens.2gm of probiotic butter mix. With 0.5gm of turmeric and 0.25ml of citric acid and addition of sodium bicarbonate in a trace amount.

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